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TITLE: Analysis of 3D Subharmonic Ultrasound Signals from Patients with Known Breast Masses for Lesion Differentiation

PRINCIPAL INVESTIGATOR: John Eisenbrey, PhD

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14. ABSTRACT ***(Maximum 200 Words)***
The purpose of this award was to help the principle investigator transition into a long term career in breast cancer imaging research through both training and independent research. Training components included regular meetings with both the physician and engineering co-advisors, observation of an NIH funded multi-center breast imaging trial using contrast-enhanced 3D subharmonic breast imaging, and attendance at radiology-based medical and engineering conferences.

The research component of this award has produced several advancements in quantitative image processing of dynamic volumetric ultrasound data. Quantitative assessments of the imaging technique (subharmonic ultrasound) were performed in year 1 to compare image quality to current state of the art techniques. Analysis of a large human data set using signal intensity over time was performed in year 2 to better identify vascular morphology in breast masses. Finally, these curves were utilized in year three to quantify blood flow kinetics to better characterize breast masses.

The funding provided by this award mechanism has allowed the PI to develop skills necessary for breast imaging research and diversify his research interests. Resultantly, the PI has now transitioned into a career as an independent faculty researcher in the department of Radiology at Thomas Jefferson University.

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3 TABLE OF CONTENTS

4 INTRODUCTION

The purpose of this award was to help the principle investigator (PI) transition into a long term career in breast cancer imaging research through both training and independent research. At the beginning of this project, the primary focus was on the training component. The training portion of this grant consisted of both clinical and engineering components. The engineering training component focused on an image processing curriculum under the guidance of the project mentor. The clinical training component included observation of an NIH funded, multi-center breast imaging trial (data of which was used in the research portion of this grant), time spent with radiologists specializing in breast imaging, and attendance at clinical research and case conferences.

As the project progressed, emphasis was shifted more towards the research component of the grant. This research included the development of computer-based analysis software that extracted physical parameters from a new method of ultrasound imaging (subharmonic imaging) to improve breast lesion characterization. These algorithms were also applied to an existing contrast enhanced ultrasound dataset from murine xenografts to determine their relationship with immunohistochemical angiogenic marker expression (which may be useful as a potential tool for monitoring treatment response). Currently, mammography leads to an unacceptably high rate of false positive findings. Thus, the ultimate goal of this research was to develop subharmonic ultrasound image (SHI) processing algorithms to improve the classification of breast lesions.

The funding provided by this award mechanism has also allowed the PI to develop skills necessary for breast imaging research and diversify his research interests. Resultantly, the PI has now transitioned into a career as an independent faculty researcher in the department of Radiology at Thomas Jefferson University.

5 BODY

5.1 Training Component

The training component of this research has been split into breast imaging and image processing arms. The PI had the opportunity to observe radiologists at TJU's breast imaging center as they interpret mammograms, breast ultrasounds, and breast MRIs. Learning within the clinical environment was also augmented by working through the texts: Kopan's Breast Imaging, and Stravos' Breast Ultrasound under the guidance of the project mentors to better understand current state of breast imaging. This has helped not only to understand the reading process, but also areas for potential future improvements.

Additionally, training within the breast imaging arm of this project included attending TJU's breast case conferences, weekly Kimmel Cancer Center grand rounds, Department of Radiology seminars, and attendance at the 2011-2013 Leading Edge in Diagnostic Ultrasound Breast Ultrasound Tutorial. These presentations have helped the PI gain a larger picture of breast cancer care and emerging areas of research within the field.

Due to the PI's background in engineering the image processing training arm required for this project was less inclusive. Originally, the training curriculum was to include enrollment in the University of Pennsylvania's Graduate Biomedical Image Processing course (UPenn CIS 537- Biomedical Image Analysis). However, due to the timing and release of the award, enrollment in this Fall course was not possible. Instead, the PI and project mentor found it suitable to replace this course with a 3 day Matlab programming training course and a 2 day image processing course both offered by Mathworks (Nattick, MA). These courses not only fit within the required time window, but also dealt exclusively with Matlab (the programming language that will be used for the research component of this project). Additionally, the PI attended biomedical image processing webinars offered by Matlab to further enhance this skill set. This has greatly helped the PI prepare for the research project focusing on image processing of breast lesions.

Work within the training arm also included observation of an NIH funded multi-center breast imaging trial using contrast-enhanced 3D subharmonic imaging for the characterization of mammographically identified breast masses. The PI's involvement in the project included assistance with drafting the protocols, gaining regulatory approval, testing the experimental software, observing data collection, and data analysis. To date, 158 patients have been observed at Thomas Jefferson University (TJU) and patient enrollment began in late September 2012 at the University of California, San Diego (51 subjects enrolled to date). The ability to assist in this process allowed the PI to gain a better understanding of the requirements for starting and running a large scale breast imaging trial. Additionally the data collected was used for algorithm development as discussed in the research component.

5.2 Research Component

Year 1

The original datasets used for the research component of this project were collected from patients with mammographically identified breast lesions as part of NIH R01 CA140338. The goal of this NIH-funded research is to utilize the improved ability of subharmonic imaging (SHI) to visualize ultrasound contrast agent due to SHI's improved overall tissue suppression relative to current commercially available imaging techniques. Figure 1 shows an example of this data, presenting images from a patient's lesion under A) grayscale B-mode, B) color Doppler without contrast, C) Rendered 4D harmonic imaging (HI) prior to contrast injection, D) Rendered 4D HI during contrast wash-in, E) Rendered 4D SHI prior to contrast wash-in, and F) Rendered 4D SHI during contrast wash-in. As shown by Doppler imaging (Fig. 1B), little flow is located within the actual lesion, although a blood vessel is visualized just to the left (denoted by white arrow). No contrast was detected by HI as shown by the nearly identical images in Fig. 1C and D, presumably due to the high levels of tissue signal apparent throughout the sequence. While little change is observed within the lesion between the pre and post contrast SHI images, the previously identified vessel to the left shows improved enhancement and connectivity within an area of multiple bifurcations. Visualization of these blood vessels would not be possible without the improved levels of tissue suppression afforded in SHI.

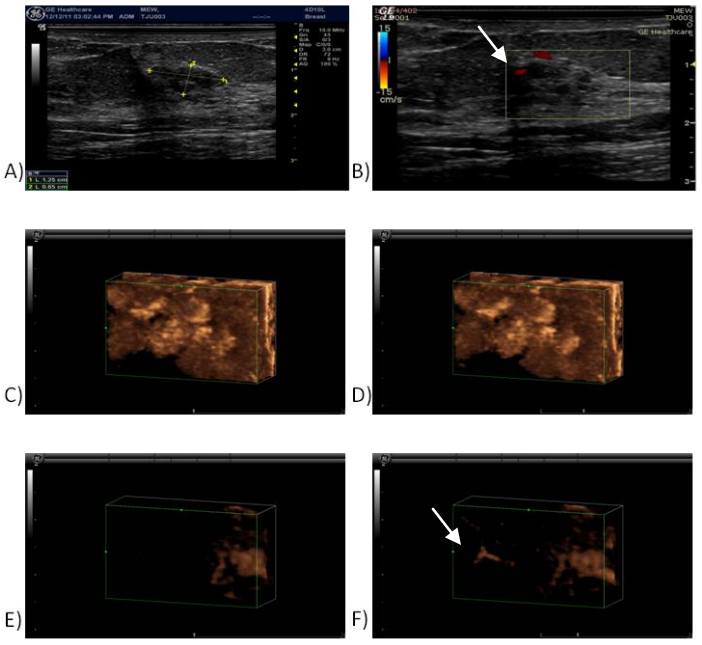

Figure 1. Study showing A) grayscale B-mode with size measurements, B) color Doppler without contrast, C) Rendered 4D HI prior to contrast injection, D) Rendered 4D HI during wash-in, E) Rendered 4D SHI prior to wash-in, and F) Rendered 4D SHI during wash-in.

While these improvements with SHI are evident from the images above, quantification of SHI's improvement relative to HI was required. Using data obtained from an *in vitro* flow phantom and renal scanning of canines from a previous optimization study (the data of which was made available to the PI), contrast to tissue ratios (CTR) were calculated. These ratios provide a quantifiable indicator of a physician's ability to differentiate contrast agent from the surrounding tissue and are calculated as:

$$CTR = \frac{2(\gamma_V - \gamma_T)^2}{\sigma_V^2 + \sigma_T^2},$$

where γ_V and γ_T represent the mean backscatter signal strength in the vessel and tissue respectively; and σ^2_V and σ^2_T represent the variance in the respective ROIs [1]. Based on previous optimization work from an *in vitro* flow phantom and canines, we found SHI resulted in significant improvement in CTR levels relative to HI both *in vitro* (12.11±0.52 vs. 2.67±0.77, $p<0.001$) and *in vivo* (5.74±1.92 vs. 2.40±0.48, $p=0.04$) [2]. This work was not only useful in quantifying SHI's relative improvements, but also provided a quantifiable metric for assessing future image processing improvements.

In order to augment the improvements offered by SHI over the current HI industry standard, parametric imaging algorithms were used to further improve these results. Approval to proceed with the processing of this data was received by both TJU's Institutional Review Board and the U.S. Army Medical Research and Materiel Command, Office of Research Protections, Human Research Protection Office, who ruled that further processing of these images originally obtained for research purposes did not constitute human research.

Preliminary algorithms were then constructed to process these datasets in order to better visualize the lesion vasculature and blood flow parameters. These parametric maps

include maximum intensity projections (MIP), time to peak intensity, perfusion (based on the rate of contrast wash in), and time integrated intensity, which correlates to net blood flow over the contrast wash in cycle. These algorithms have been modified and applied for both 2D compressed representations of the volumes and over the entire volume space. Figure 2 shows an example of MIP processing from data obtained from the case shown in In Fig. 2A, an example of the raw, unprocessed data at midline reveals very little of the total vasculature. Figure 2B shows the MIP of this midline slice over the entire contrast sequence, while Figure 2C shows the volumetric (3D volume compressed to a 2D place) MIP at contrast wash in. Finally, Figure 4D shows both the temporal and volumetric MIP. This processing resulted in noticeable enhancement of the vascular structures.

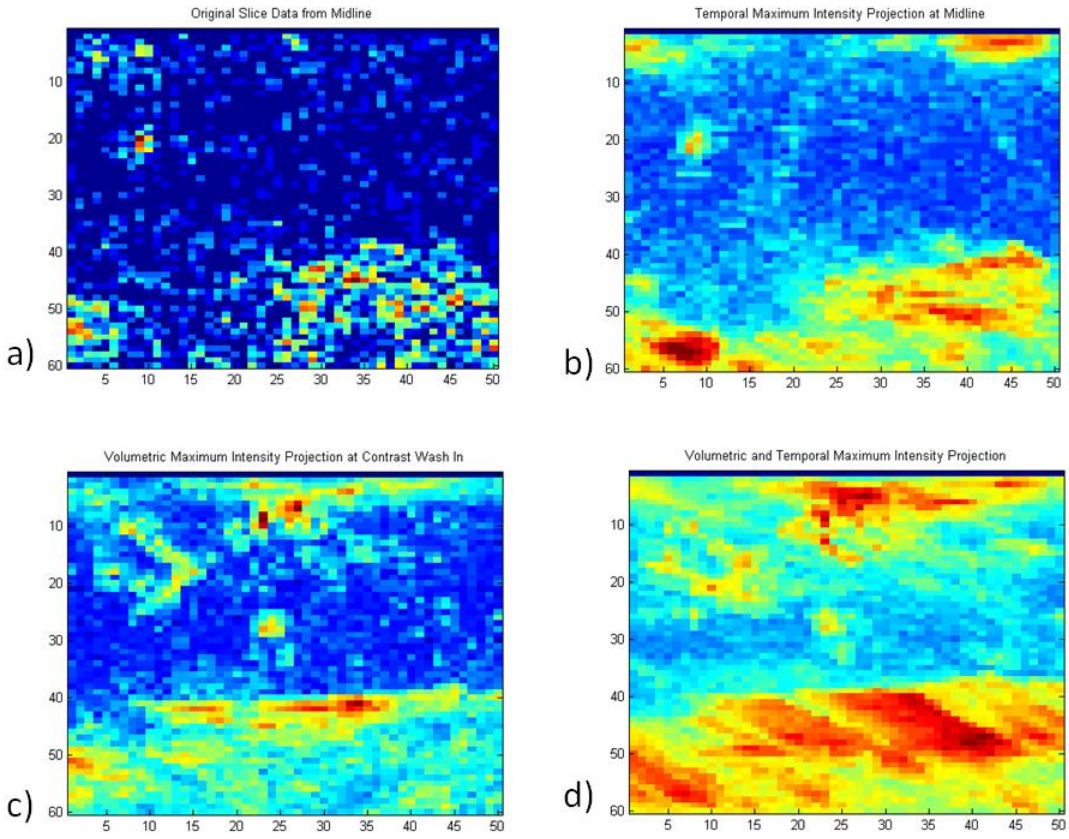

Figure 2. Study showing A) unprocessed slice data at midline from case presented in Figure 1, B) temporal maximum intensity projection at mid line slice, C) Volumetric maximum intensity projection during contrast wash in, and D) Temporal and volumetric maximum intensity projection.

These results were a first iteration of algorithms, but still provide improved depiction of lesion vasculature relative to the unprocessed images.

Year 2

The main focus of the research component in year 2 was the development of methods to quantify and visualize blood flow kinetics from subharmonic signals within breast lesions and to improve the visualization of blood vessels in 3D subharmonic ultrasound volumes. Image processing focused on isolating regions of contrast flow in the three dimensional space to better depict these regions. Additionally, these measurements and their respective standard deviations were used as a potential quantifiable parameter for separating malignant vs. benign lesions. Time intensity curves (TICs) were generated for each individual slice for each clinical case. This allows visualization of changes in signal intensity over the contrast agent wash in and wash out (an indicator of lesion vascularity). An example of this process is shown in Figure 3. A benign lesion with highly ordered, centralized flow is shown in the top portion of figure 3, while a malignant, invasive mammary carcinoma with vascularity throughout the lesion is shown in the bottom portion of the figure. When performing these algorithms over the entire dataset, we found that benign lesions showed a statistically significant difference in changes in signal intensity between the central and peripheral areas of the tumor (1.83 dB vs. 1.15 dB, $p < 0.001$), while no significant difference was observed between the core and peripheral regions in malignant lesions. These results are consistent with the concept that malignant lesions will have a highly unorganized, diffuse blood supply, while benign

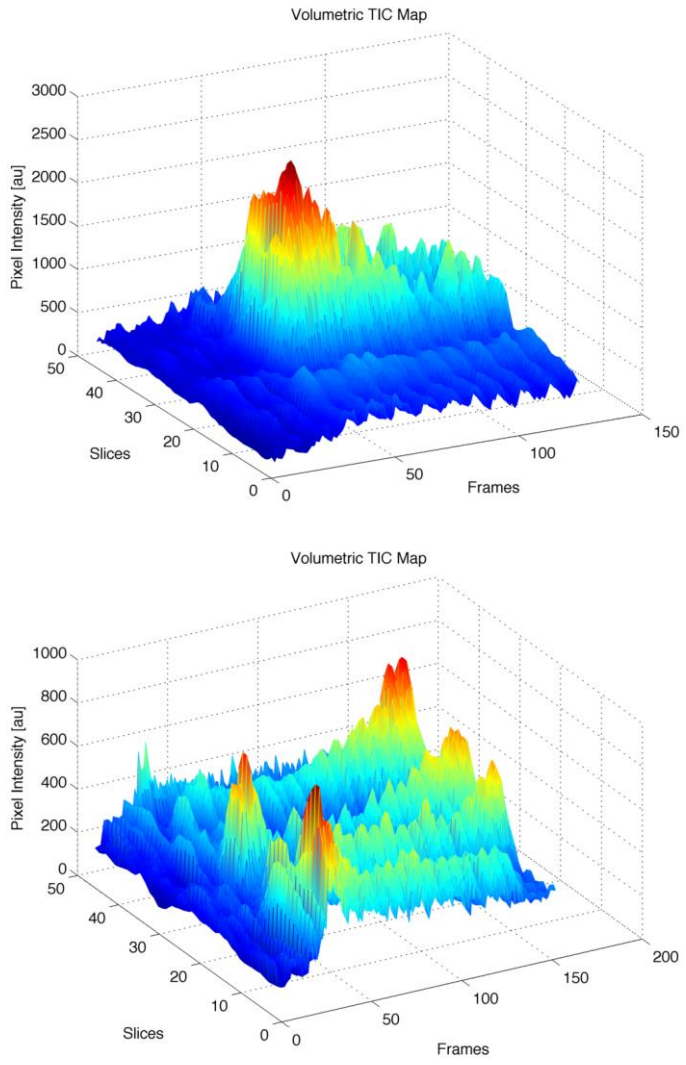

Figure 3. Signal intensity over contrast wash in and wash out at each individual slice from a benign lesion (top) and a case of high grade, invasive mammary carcinoma. Notice that while the vascularity (shown by change in signal intensity) within the benign lesion is based primarily within a core region, vascular locations are highly chaotic with a larger deviation in the malignant case.

lesions are more likely to have structured, central vascularity.

Previously, we showed preliminary results of algorithms for parametric imaging of ultrasound contrast agent wash-in as a means for modeling blood flow kinetics and thus, potentially becoming a useful quantifiable parameter for diagnosis [3]. The project mentor (Dr. Forsberg) previously explored the use of contrast-enhanced ultrasound peak intensity to predict tumor angiogenic marker expression in two subcutaneous tumor models in rats. Such accurate predictive markers could offer a noninvasive technique for monitoring treatment response in breast cancer therapy. As part of the research portion of this project, the parametric algorithms in development for subharmonic imaging were applied to this existing dataset of contrast enhanced ultrasound exams of subcutaneous tumors in rats.

As part of the original study, breast tumor (NMU) or glioma (C6) cells were implanted in either the abdomen or thigh of 144 rats. After 6, 8 or 10 days, rats received a bolus ultrasound contrast agent (UCA) injection of Optison (GE Healthcare, Princeton, NJ; 0.4ml/kg) during power Doppler imaging (PDI), HI, and microflow imaging (MFI) using an Aplio ultrasound scanner with 7.5 MHz linear array (Toshiba America Medical Systems, Tustin, CA). Tumors were then stained for 4 immunohistochemical markers (bFGF, CD31, COX-2, and VEGF). To evaluate the parametric code on this dataset, time-intensity curves of contrast wash-in were constructed on a pixel-by-pixel basis and averaged to calculate maximum intensity, time to peak, perfusion, and time integrated intensity (TII). Significant correlation over the entire dataset (shown below in Figure 4) was only observed between TII and VEGF for all three imaging modes (R = -0.35,-0.54,-0.32 for PDI, HI and MFI, respectively; $p<0.0001$).

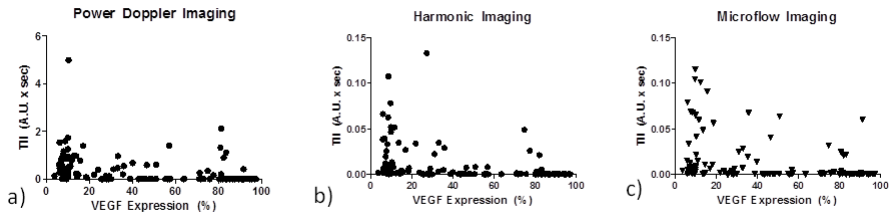

Figure 4 : Data from correlation of time integrated intensity (TII) with vascular endothelial growth factor (VEGF) expression from power Doppler (a), harmonic (b), and microflow (c) imaging.

Tumor type and location affected these correlations, with the strongest correlation of TII to VEGF found to be with implanted C6 cells (R = -0.43,-0.54,-0.52 for PDI, HI and MFI, respectively; $p<0.0002$). These results offered significant improvement in the overall correlation relative to the previous, static measurements used during the initial study. Thus, while results appear to be imaging mode and tumor type dependent, the algorithms being developed for lesion classification purposes as part of this project may also be useful for monitoring treatment response in known malignant lesions.

As a secondary research focus, we have explored photoacoustic imaging as a potential means for monitoring treatment response in breast cancer therapy. Over the course of the project the project mentor received funding to purchase a Vevo 2100 small animal ultrasound scanner with photoacoustic capabilities (through NIH S10 OD010408-01). Photoacoustics (PA) is an emerging imaging modality and these systems are now commercially available for preclinical research. Initially, light (generally in the 700-900 nm wavelength range) is directed through the tissue from a focused, tunable laser. As the light is absorbed, it generates thermal expansion which can then be detected using an ultrasound transducer. This technique benefits from the specificity of optical imaging combined with both the resolution and increased penetration depths of ultrasound.

MDA-MB-231 breast cancer tumors were implanted in the mammary pad of 11 nude rats. Ultrasound and photoacoustic (PA) scanning was performed using the Vevo2100 scanner. Contrast-enhanced ultrasound (CEUS) was used to create MIPs as a measure of tumor vascularity. Photoacoustics were used to determine hemoglobin signal (HbT), oxygenation levels in detected blood (SO2 Avg), and oxygenation levels over the entire tumor area (SO2 Tot). An example of these images are shown in Figure 5, demonstrating strong photoacoustic signal (top) with quantitative oxygenation measurements, grayscale ultrasound of the tumor (bottom left), and the maximum intensity UCA signal used as a measure of vascularity (bottom right). Tumors were then stained for vascular endothelial growth factor (VEGF), Cyclooxygenase-2 (Cox-2), and the platelet endothelial cell adhesion molecule CD31. Correlations between findings were analyzed using Pearson's coefficient. Significant correlation was observed between CEUS derived vascularity measurements and both PA indicators of blood volume (R = 0.61 for HbT, R = 0.64 for S02 Tot). However, no significant correlation was observed between these measurements and any of the immunohistochemical markers (p>0.18). SO2 Avg showed significant

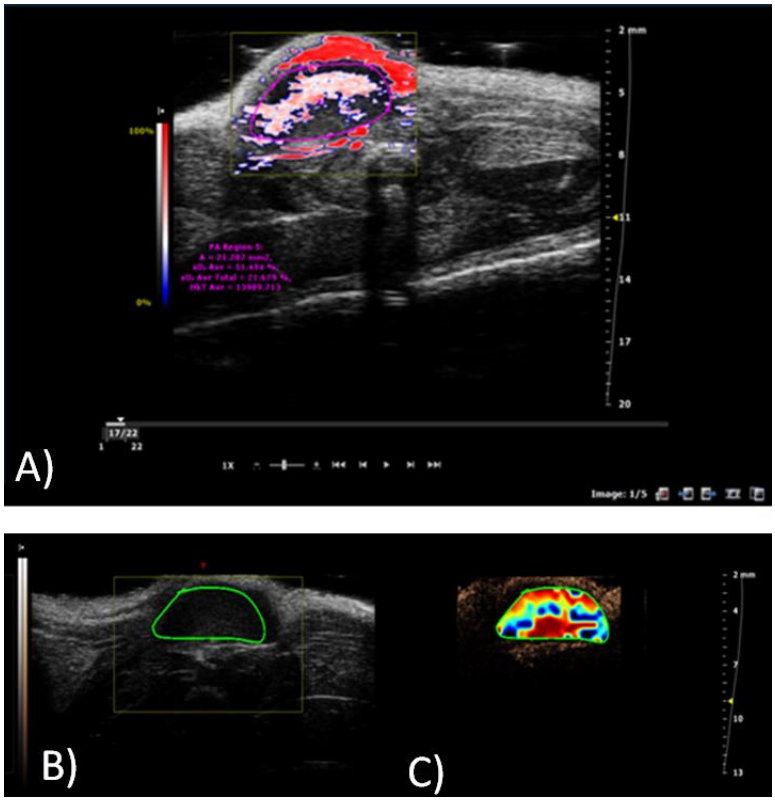

Figure 5. Imaging example with tumor ROI outlined. A: Representative image of a tumor in PA mode with high hemoglobin signal (13,989) and a relatively high SO2 Avg (51.5%). B: Corresponding CEUS image post contrast and C: peak enhancement parametric image.

inverse correlation with Cox-2 (R = -0.65, p=0.03), but not VEGF or CD31 (p>0.5). While photoacoustically derived HbT and SO2 Tot may be a good indicator of tumor fractional vascularity, SO2 Avg appears to be a better predictor of Cox-2 expression. This research appears to indicate photoacoustics may be a better tool for monitoring breast cancer treatment response on the molecular level and may be a future area of research interest for the PI.

Year 3

The majority of research in year 3 has focused on applying algorithms developed in year 2 to our large clinical dataset of 4D HI and SHI contrast-enhanced ultrasound exams. In addition, the PI transitioned to a role as an independent faculty member in the Radiology Department during year three, with a focus on breast imaging.

All image processing was performed in MATLAB (2012a, The Mathworks, Natick, MA). Initially, a radiologist and an US physicist (in consensus) analyzed the contrast-enhanced 3D HI and 3D SHI volumes (all planes included) to identify lesions with UCA flow. If any single plane showed UCA activity, a ROI was selected corresponding to the visualized vascularity in 4D View. If none of the planes in the volume showed flow, then no ROI was designed. Both the 3D rendered image as well the individual slice information were used to identify UCA flow. This ROI was projected across the entire lesion volume. The ROI was then mapped on to the raw volumetric 3D slice data extracted in MATLAB. TICs were then generated for all slices within the ROI of the imaged volume (as described above under year 2). The TIC map was used to develop an averaged volumetric background template based on the first 3 volumes (range; 1-3 seconds) of data.

The volumetric background template was subtracted from the entire volume sequence in order to reduce any residual background tissue signals (as described above in year 1). . The background filtered TIC map was then used to identify key time-points corresponding to UCA flow kinetics; specifically, baseline (T_B: time-point corresponding to 10% of peak UCA intensity), peak UCA intensity (T_P: time-point of peak UCA intensity) and washout (T_W: time-point of return to baseline UCA intensity). In order to analyze the vascular presence throughout the lesion volume, the normalized change in mean UCA intensity within the ROI (polyline) from T_B to T_P for each TIC in the volume was measured as a distribution across the entire volume. Subsequently, the lesion volume was split into peripheral (defined as the outer third of the entire tumor area including 2 mm around the lesion boundary) and central sections to facilitate assessment of vascular heterogeneity. In order to identify any basic difference in the vascular characteristics between malignant and benign lesions, the average UCA intensity (single value) at T_P of all slices in the volume was evaluated and compared between malignant and benign cases using an unpaired t-test.

Data was available for 134 subjects. The overall mean age of the women who participated in this study was 52 ± 13.3 years. Biopsy of lesions found 99 benign and 35 malignant cases. There was a significant difference between the mean ages of the malignant and benign cases 58 ± 11.5 vs. 50 ± 13.3 years, respectively ($p < 0.001$). In

terms of specific lesion types, invasive ductal carcinomas (23/35) made up the majority of the malignant cases, while fibroadenoma (30/99) was the most prevalent classification of the benign lesions. Vascularity was observed in 8 lesions (5 benign and 3 malignant) for 3D HI and 68 lesions (49 benign and 19 malignant) with 3D SHI. Given the low number of cases identified with UCA flow in 3D HI mode no further image processing and analysis was performed on this group.

Overall, the mean change in UCA intensity across the entire lesion volume between benign and malignant lesion groups was not significantly different (1.32 ± 0.89 vs. 1.38 ± 1.23 dB, $p = 0.73$). Instead, the vascular heterogeneity plots were developed (based on the relative change of UCA intensity from T_B to T_P for each slice within the lesion volume) in order to better quantify the variation in vascular activity. Figure 6A depicts the vascular heterogeneity plot of a benign case (a fibroadenoma) across the peripheral and central sections. The presence of vascularity in the central sections is increased compared to the peripheral sections. The vascular heterogeneity of an invasive ductal carcinoma is shown in Fig 6B. In this case, vascular activity is seen across the entire lesion volume. While a slight increase in vascular activity is seen in a few of the central slices compared to the general peripheral regions, this difference is not comparable to the variations in the benign case.

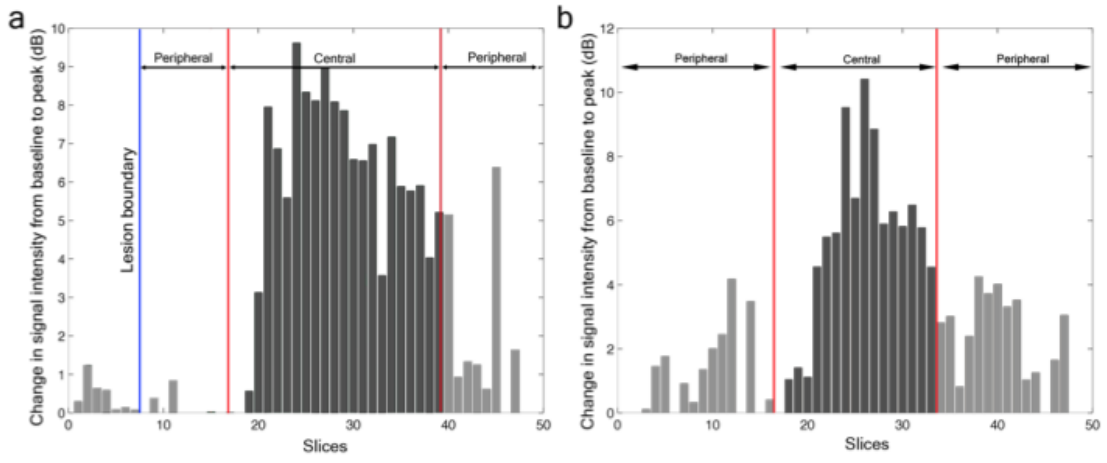

Figure 6. Vascular heterogeneity plots of (a) benign (fibroadenoma) and (b) malignant (invasive ductal carcinoma) across the peripheral and central sections of the lesion volume.

The relationship of this vascular heterogeneity is shown in Figure 7. Overall, for benign lesions, the central sections of the lesion showed significantly increased vascular activity relative the peripheral sections (1.71 ± 0.96 vs. 1.13 ± 0.79 dB, $p < 0.001$). For malignant lesions however, no significant differences were observed in the vascular activity between the central and peripheral sections (1.66 ± 1.39 vs. 1.24 ± 1.14 dB, $p = 0.24$). Thus, this analysis identifying vascular heterogeneity in 3D space using a 4D dataset may be a useful measure for distinguishing benign from malignant breast masses.

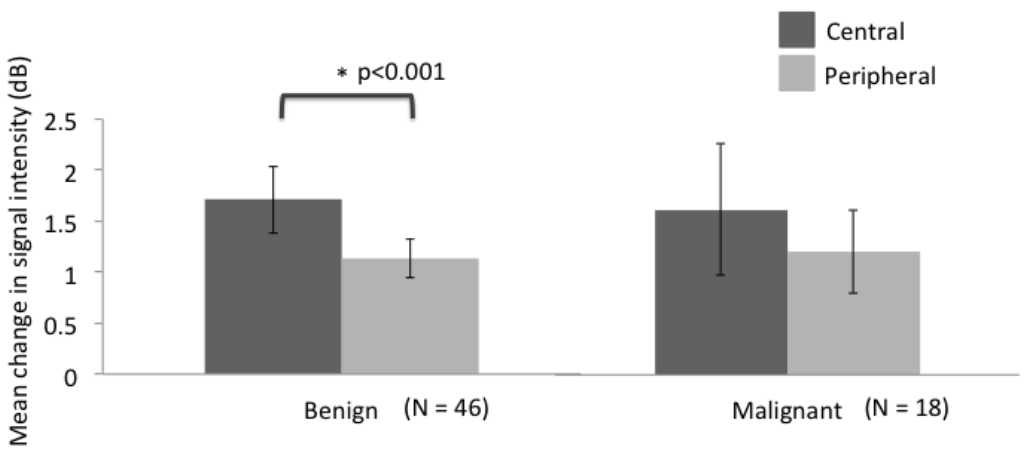

Figure 7. Overall comparison of vascularity between the central and peripheral sections for benign and malignant cases.

Data collection for the 4D subharmonic breast study (NIH R01 CA140338) is expected to continue until February 2016. Because the PI of this DoD award has transitioned to a full time, independent faculty job at Thomas Jefferson University, he will still have access to this dataset at the completion of the RO1. Thus, the parametric and vascular heterogeneity algorithms developed in this project will continue to be applied to this dataset and used to quantify biomarkers for characterizing benign from malignant breast masses. In addition, it is anticipated that these algorithms will also form the basis for future image processing projects over the course of the PI's career.

6 KEY RESEARCH ACCOMPLISHMENTS

Training Component

- Observation of patient enrollment, site setup, regulatory approval and data collection of a large, multi-center breast imaging trial
- Observation of physician reading of mammograms, breast ultrasounds and breast MRI to better understand diagnostic thought process
- Worked through Kopan's Breast Imaging, and Stravos' Breast Ultrasound to better understand current state of breast imaging
- Attendance at 2011-2013 Leading Edge in Diagnostic Ultrasound Breast Ultrasound Tutorial, American Institute of Ultrasound in Medicine 2011-2014 Annual Meeting, the 2011, 2012 and 2014 IEEE Ultrasonics Symposium, the 2013 World Molecular Imaging Congress, and the 2014 International Contrast Ultrasound Society meeting.
- Completion of courses in basic program and image processing using Matlab
- Attendance of Kimmel Cancer Center, Radiology, and Breast Cancer seminars/ case conferences

Research Component

- Quantified contrast to tissue ratios of 4D SHI to directly compare to 4D HI from optimization data
- Developed algorithms to create maximum intensity projections from 4D SHI datasets which will be used for improving visualization of blood flow
- Developed and applied algorithms to measure blood flow kinetics based on temporal data for 4D subharmonic breast ultrasound exams.
- Applied these algorithms to an existing data set of contrast enhanced ultrasound exams in murine tumor xenografts to determine their ability to predict angiogenic marker expression
- Investigated spatial deviations in subharmonic derived blood flow parameters as a potential diagnostic criteria of malignancy
- Began preliminary studies into the use of photoacoustics as a potential means of evaluating breast tumor vascularity and immunohistochemisty in a murine model.

7 REPORTABLE OUTCOMES

Publications:

1. J.R. Eisenbrey, A. Sridharan, P. Machado, V.G. Halldorsdottir, J.K. Dave, J.B. Liu, S. Park, S. Dianis, K. Wallace, K.E. Thomenius, F. Forsberg. 3D subharmonic imaging in vitro and in vivo. *Acad. Radiol.* 2012; 19:732-739.

2. J.R. Eisenbrey, C.C. Wilson, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Correlation of ultrasound contrast agent derived blood flow parameters with immunohistochemical angiogenesis markers in murine xenograft tumor models. *Ultrasonics*, 2013; 53:1384-91.

3. A. Sridharan, J.R. Eisenbrey, P. Machado, J.B. Liu, V.G. Halldorsdottir, J.K. Dave, H. Zhao, Y. He, S. Park, K. Wallace, K.E. Thomenius, F. Forsberg. Perfusion estimation using contrast enhanced three-dimensional subharmonic ultrasound imaging: an in vivo study. *Investigative Radiology,* 2013; 48:654-60.

4. J.R. Eisenbrey, A. Marshall, D.A. Merton, J.B. Liu, T.B. Fox, A. Sridharan, F. Forsberg. Comparison of photoacoustically derived hemoglobin and oxygenation measurements with contrast enhanced ultrasound estimated vascularity and immunohistochemical staining in a breast cancer model. *Ultrason Imaging*, vol. 37, no 1, pp. 42 – 52, 2015.

5. J.R Eisenbrey, A. Sridharan, J.B. Liu, F. Forsberg. Recent experiences and advances in contrast-enhanced subharmonic ultrasound. *Biomed Res Int.* In Press.

6. A. Sridharan, J.R. Eisenbrey, P. Machado, H. Ojeda-Fournier, A. Wilkes, A. Sevrukov, R.F. Mattrey, K. Wallace, C.L. Chalek, K.E. Thomenius, F. Forsberg. Quantitative analysis of vascular heterogeneity in breast lesions using contrast-enhanced three-dimensional harmonic and subharmonic ultrasound imaging.

Submitted to *IEEE Transactions on Ultrasonics, Ferroelectrics, and Frequency Control* December 2014.

Abstracts and Conference Proceedings:

1. J.R. Eisenbrey, J.K. Dave, V.G. Halldorsdottir, A. Sridharan, S. Park, S. Dianis, D.A. Merton, P. Machado, J.B. Liu, J.M. Gonazlez, C. Miller, K.E. Thomenius, D.B. Brown, V. Navarro, F. Forsberg. Simultaneous B-mode/subharmonic imaging and 3D subharmonic imaging on a modified commercial ultrasound scanner. *Proc. IEEE Ultrason. Symp.*, 624-627, 2011.

2. J.R. Eisenbrey, A. Sridharan, D.A. Merton, P. Machado, V.G. Halldorsdottir, J.K. Dave, J.B. Liu, H. Zhao, S. Park, S. Dianis, C.L. Chalek, K.E. Thomenius, F. Forsberg. In vitro and in vivo 4-dimensional subharmonic imaging. *Proc. AIUM Annual Meeting*, J. Ultrasound Med. 31:S50, 2012.

3. C.C. Wilson, J.R. Eisenbrey, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Parametric imaging of ultrasound contrast shows an improved correlation with immunohistochemical markers in a glioma model compared to nonparametric imaging. *Proc. AIUM Annual Meeting*, J. Ultrasound Med. 31:S105, 2012.

4. J.R. Eisenbrey, A. Sridharan, P. Machado, D.A. Merton, J.B. Liu, K. Wallace, S. Park, S. Dianis, C.L. Chalek, K.E. Thomenius, F. Forsberg. 4D Subharmonic Imaging In Vivo. *Proc. IEEE Ultrason. Symp.*, 2012.

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2. C.C. Wilson, J.R. Eisenbrey, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Parametric imaging of ultrasound contrast shows an improved correlation with immunohistochemical markers in a glioma model compared to nonparametric imaging. AIUM Annual Meeting, Phoenix Arizona, March 31'st 2012.

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4. J.R. Eisenbrey, D.A. Merton, J.B. Liu, T.B. Fox, A. Sridharan, F. Forsberg. Ultrasound contrast agent based vascularity measurements versus photoacoustic derived hemoglobin and oxygenation measurements in a breast cancer model. 2013 World Molecular Imaging Congress, Savannah, GA, September 2013.

5. J.R. Eisenbrey, C.C. Wilson, A. Sridharan, R.J. Ro, T.B. Fox, J.B. Liu, S.Y. Chiou, F. Forsberg. Prediction of VEGF expression in two tumor models using dynamic contrast enhanced ultrasound: identification of optimal imaging mode and temporal parameter. 2013 World Molecular Imaging Congress, Savannah, GA, September 2013.

6. J.R. Eisenbrey, A. Sridharan, D.A. Merton, P. Machado, K. Wallace, C.L. Chalek, H. Ojeda-Fournier, R.F. Mattrey, K. Thomenius, F. Forsberg. 4D subharmonic breast imaging. The Leading Edge in Diagnostic Ultrasound Annual Conference. Atlantic City, NJ, May 2013.

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9. J.R. Eisenbrey. Contrast enhanced ultrasound. Department of Orthopaedic Surgery Seminar. Thomas Jefferson University, November 11th, 2013.

10. J.R.Eisenbrey, D.A. Merton, J.B. Liu, A. Marshall, T.B. Fox, A. Sridharan, F. Forsberg. Comparing photoacoustic derived hemoglobin and oxygenation measurements and ultrasound contrast agent derived vascularity measurements with immunohistochemical staining in a breast cancer xenografts model. 2013 RSNA Annual Meeting, Chicago, IL, December 2013.

11. J.R. Eisenbrey, A. Sridharan, D.A. Merton, P. Machado, K. Wallace, C.L. Chalek, H. Ojeda-Fournier, R.F. Mattrey, K. Thomenius, F. Forsberg. 3D subharmonic breast imaging- an update. The Leading Edge in Diagnostic Ultrasound Annual Conference. Atlantic City, NJ, May 2014.

12. J.R. Eisenbrey, P. Machado, A. Sridharan, H. Ojeda-Fournier, A. Wilkes, A. Sevrukov, R.F. Mattrey, F. Forsberg. 4D Harmonic and Subharmonic Contrast-Enhanced Ultrasound for the Characterization of Breast Masses: Update on a Multi-center Prospective Study. IEEE Ultrasonics Symposium, Chicago IL, September 4th, 2014.

Awards and Honors:

1. 2012 AIUM scientific poster 2nd place for 'Parametric imaging of ultrasound contrast shows an improved correlation with immunohistochemical markers in a glioma model compared to nonparametric imaging'

2. Thomas Jefferson University Hospital Department of Radiology Judy Dubbs Memorial Research Award.

3. GE "Fresh Face Program" Award Winner- International Contrast Enhanced Ultrasound Society Annual Meeting. Chicago September 2012.

4. Nominated for Best Poster Award, 2013 World Molecular Imaging Congress, Savannah, GA.

5. Student Travel Award for 2013 World Molecular Imaging Congress, Savannah, GA.

6. Drexel University, School of Biomedical Engineering, Science and Health Systems, 2014 Distinguished Alumni Award 2014

8 CONCLUSIONS

The training components in the project have provided the PI with a better clinical understanding of breast imaging and helped him develop the necessary image processing skills for an independent career in breast radiology research. Experimental algorithms have been developed to quantify vascularity, heterogeneity, and blood flow kinetics in

volumetric contrast-enhanced ultrasound exams. Such algorithms are expected to be helpful for the classification of masses identified on mammography. These algorithms have also been investigated as a potential sign of angiogenic marker expression. Finally, this award has also allowed the PI to successfully transition into a role as an independent radiology faculty member with a research focus on breast imaging.

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